Support for Amendments

Applicants have amended the specification to update the status of all cited U.S. patent applications and to correct a typographical error that occurs in Figure 6 and the legends to Figures 5 and 6. Support for correction of this typographical error is found in the specification, for example, at page 16, lines 20 and 21, page 17, lines 1 and 2, and page 29 lines 12-13. An equivalent correction to the typographical error has been made to Figure 6. Typographical errors have also been corrected and clarifying amendments have been made to the specification at pages 15, 22-29, 36-37, and 41.

In addition, claims 40-42, 51, 52, 57, 59-61, and 63-64 have been amended. Claims 40, 41, and 59 have been amended to include the limitations of claims 46, 47, and 50 (which claims are now canceled), and to further specify that the claim is directed to screening or detecting methods that make use of one or a library of scaffold-based proteins that include members having the ability to bind compounds as a result of randomizing at least three loops. These amendments find support in the specification at page 20, line 19 through page 22, line 5, and page 4, lines 8-10, respectively. The amendment to claim 51 finds support in the specification, for example, at page 16, lines 5-8. New claim 68 finds support in the specification, for example, at page 17, line 23 - page 18, line 3. The remaining claim amendments merely change claim dependencies or bring the language of the dependent claims into conformity with the language of amended independent claims 40, 41, and 59.

No new matter is added by any of these amendments.

Applicants reserve the right to pursue all canceled subject matter in this or any currently pending or future related application.

Objection to the Drawings

As required, Applicants submit herewith new Figures 1-17. These drawings correct the errors recited in the recent Form PTO-948. This objection may be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 40-67 stand rejected, under 35 U.S.C. § 112, second paragraph, as being indefinite. This rejection is based first on the assertion that it is "unclear as to what sequences are considered randomized since the claims do not contain a template sequence with which to compare." The Office goes on to suggest that "[A]ddition of a template sequence with which to determine if a loop was randomized would clarify this matter." As recommended by the Office, Applicants have amended the claims to specify that the claimed proteins are scaffold-based proteins and that the scaffold is the tenth module of the human fibronectin type III domain (^{10}Fn3), the template sequence of which was known in the art at the time of filing (see the specification at page 8, lines 14-18) . In view of this amendment, this basis for the rejection may be withdrawn.

In addition, the term "randomized" in claims 40-42 and 59 is rejected as unclear

based on the assertion that this term is more appropriately used when describing a library of proteins. In response to this rejection, the Office is first directed to Applicants' specification at page 9, lines 1-2, where the term "randomized" is defined:

> By "randomized" is meant including one or more amino acid alterations relative to a template sequence.

In view of this definition, Applicants believe the term to be definite. In addition, Applicants note that claims 40 and 42 are now directed to the screening of a library of proteins. This basis for the rejection may therefore also be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 40-67 stand further rejected, under 35 U.S.C. § 112, first paragraph on the basis that the specification does not provide a written description for the claimed invention, nor does the specification reasonably provide enablement for that invention. As applied to the present claims, this rejection is respectfully traversed.

I. Written Description

Claims 40-67 first stand rejected as failing to provide an adequate written description for the claimed invention. This rejection turns on the assertion that the claims cover "any protein binding method wherein the protein used in the method contains at least one loop. The claims encompass methods using proteins having any binding activity or enzymatic activity and almost any structure (in addition to the "at least one

randomized loop")."

The Office cites the Guidelines for Examination of Patent Applications under the Written Description Requirement. Applicants point out that those Guidelines indicate that the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species, by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics (e.g., structural, physical, and/or chemical properties), by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show an Applicant was in possession of a claimed genus. This standard is met for the present claims.

Claim 40 and its dependent claims, as amended, are directed to methods that make use of scaffold-based proteins wherein the scaffold is derived from "the tenth module of the human fibronectin type III domain (^{10}Fn3)" "having at least one randomized loop" where the proteins are "characterized by their ability to bind to compounds that are not bound by said human fibronectin type III domain" and where the compound binding ability results from the "randomization of said at least one loop." In claim 40, the method covers obtaining a protein having three randomized loops that binds a compound, and, in claims 41 and 59, the method covers obtaining or detecting a compound that binds to a protein having at least three randomized loops. Each of these claims satisfies the written description requirement.

In particular, actual reduction to practice of an exemplary library of proteins that may be utilized in the method of claim 40 is described in the present specification at pages 22-28. The proteins of this library include the tenth module of the human fibronectin type III domain having randomized loops. The specification also describes actual reduction to practice of a screening method that makes use of a combination library that includes three-randomized loop, two-randomized loop, and one-randomized loop species; this library was used for the selection of proteins that bound to TNF-α, a protein not bound by human ^{10}Fn3. These results are described in the specification at pages 28-29 and 36-38.

Moreover, all of the current claims and the specification supporting these claims do, in fact, include "relevant, identifying characteristics" of the scaffold-based proteins that are used in the screening and detection methods, these characteristics being both structural and functional in nature. In particular, the specification states and the claims require that the proteins include a scaffold derived from the tenth module of the human fibronectin type III domain. This domain possesses a well known scaffold structure (as shown, for example, in Figure 3), and also possesses three loops that can be randomized, thereby providing the functional characteristic of binding activity to non-fibronectin target proteins. As stated by Applicants at page 3, line 4 - page 4, line 1 of the present specification:

> The present invention provides a new family of proteins capable of evolving to bind any compound of interest. These proteins, which make use of a

fibronectin or fibronectin-like scaffold, function in a manner characteristic of natural or engineered antibodies (that is, polyclonal, monoclonal, or single-chain antibodies) and, in addition, possess structural advantages. Specifically, the structure of these antibody mimics has been designed for optimal folding, stability, and solubility, even under conditions which normally lead to the loss of structure and function in antibodies.

These antibody mimics may be utilized for the purpose of designing proteins which are capable of binding to virtually any compound (for example, any protein) of interest. In particular, the fibronectin-based molecules described herein may be used as scaffolds which are subjected to directed evolution designed to randomize one or more of the three fibronectin loops which are analogous to the complementarity-determining regions (CDRs) of an antibody variable region. Such a directed evolution approach results in the production of antibody-like molecules with high affinities for antigens of interest. In addition, the scaffolds described herein may be used to display defined exposed loops (for example, loops previously randomized and selected on the basis of antigen binding) in order to direct the evolution of molecules that bind to such introduced loops. A selection of this type may be carried out to identify recognition molecules for any individual CDR-like loop or, alternatively, for the recognition of two or all three CDR-like loops combined into a non-linear epitope.

This description is reiterated and expanded in the specification at page 13, line 20 - page 14, line 21, where it states:

The novel antibody mimics described herein have been designed to be superior both to antibody-derived fragments and to non-antibody frameworks, for example, those frameworks described above.

The major advantage of these antibody mimics over antibody fragments is structural. These scaffolds are derived from whole, stable, and soluble structural modules found in human body fluid proteins. Consequently, they exhibit better folding and thermostability properties than antibody fragments, whose creation involves the removal of parts of the antibody native fold, often exposing amino acid residues that, in an intact antibody, would be buried in a hydrophobic environment, such as an interface between variable and constant domains. Exposure of such hydrophobic residues to solvent increases the likelihood of aggregation.

In addition, the antibody mimics described herein have no disulfide bonds, which have been reported to retard or prevent proper folding of antibody fragments under certain conditions. Since the present scaffolds do not rely on

disulfides for native fold stability, they are stable under reducing conditions, unlike antibodies and their fragments which unravel upon disulfide bond breakdown.

Moreover, these fibronectin-based scaffolds provide the functional advantages of antibody molecules. In particular, despite the fact that the ^{10}Fn3 module is not an immunoglobulin, its overall fold is close to that of the variable region of the IgG heavy chain (Figure 2), making it possible to display the three fibronectin loops analogous to CDRs in relative orientations similar to those of native antibodies. Because of this structure, the present antibody mimics possess antigen binding properties that are similar in nature and affinity to those of antibodies, and a loop randomization and shuffling strategy may be employed in vitro that is similar to the process of affinity maturation of antibodies in vivo.

In view of the above, it is clear that the present specification sets forth a written description for the presently claimed screening and detection methods that make use of proteins possessing the structural and functional characteristics of the tenth module of the human fibronectin type III domain having randomized loops that result in binding to non-fibronectin targets. The written description rejection of claims 40-67 should be withdrawn.

II. Enablement

Claims 48-50 stand further rejected, under 35 U.S.C. § 112, first paragraph on the basis that the "specification, while being enabling for a method of obtaining a protein or compound which binds a protein or compound comprising contacting a protein comprising a fibronectin type III domain having at least one randomized loop under conditions that allow complex formation and obtaining the protein or compound from the complex, does not reasonably provide enablement for such a method wherein the

compound binding is mediated by one, two, or three loops of the tenth module of the fibronectin type III domain. The specification has not taught how to practice the claimed method wherein the number of loops that are involved in binding can be predicted or controlled" (emphasis added).

This rejection is moot in view of the cancellation of dependent claims 48-50. Moreover, Applicants have incorporated limitations into independent claims 40, 41, and 59 that do not require that compound binding be "mediated" by the randomized loops. Rather, the claims indicate that the new compound binding ability results from the loop randomization. No reference to "mediated by" remains in the claims. This rejection may be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 40-47, 49, 52, 59, and 66 stand further rejected, under 35 U.S.C. § 102(a), as being anticipated by Koide et al. (J. Mol. Biol. 284:1141-1151 (1998)). This rejection is respectfully traversed.

To support a rejection of a claim under § 102, a single prior art reference must describe all of the elements and limitations of the rejected claim. *Scripps Clinic & Research Foundation v. Genentech, Inc.*, 927 F.2d 1565, 18 U.S.P.Q.2d 1001, 18 U.S.P.Q.2d 1896 (Fed. Cir. 1991). Koide does not meet this standard in supporting a rejection of the present claims.

As an initial matter, Applicants note that only the abstract of this reference was available (on the internet) prior to the filing date of Applicants' parent application, filed December 10, 1998. Applicants provide herewith the publication citation available from the MEDLINE database, demonstrating that the full journal article was not published until December 11, 1998. Thus, only the Koide abstract may be properly cited against claims that have priority based on the December 10, 1998 application under 35 U.S.C. § 102(a).

In addition, Applicants note that independent claims 40, 41, 59, and dependent claims 42-47, 49, 52, and 66, now include a limitation equivalent to original claim 50, a claim determined by the Office to be free of the prior art rejections. Indeed, the currently claimed invention was not disclosed in either the abstract or the full Koide reference. The current claims are directed to screening or detection methods that make use of one or a library of human ^{10}Fn3 scaffold-based proteins wherein what is covered by these claims involves binding activity with proteins having at least three randomized loops. Koide does not teach these methods. The Koide abstract does not disclose using as a scaffold either the tenth domain or a human species of Fn3. Furthermore, the Koide publication merely discloses proteins that include a fibronectin type III domain containing mutations at particular positions in <u>two loops</u> and that bind a ubiquitin target protein. Koide does not teach or suggest that at least three loops of the protein could or should be randomized to result in target compound binding. In fact, if anything, Koide teaches that issues of

protein structure and stability counter-indicate randomization of Fn3 sequences outside of the two BC and FG loops.[1] Koide therefore does not anticipate the current claims, which require compound binding to proteins having at least three-randomized loops, and this basis for the § 102 rejection may be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 40-41, 44-46, 56-57, and 59-61 stand rejected, under 35 U.S.C. § 103, as being unpatentable over Main et al. (Cell 71:671-678, 1992) in view of Lee et al. (Protein Engineering 6:745-754, 1993) and Nygren et al. (Curr. Opin. Struct. Biol. 7:463-469, 1997). As applied to the current claims, this rejection is also respectfully traversed.

As discussed above, the current claims are directed to screening or detection methods that make use of one or a library of human ^{10}Fn3 scaffold-based proteins wherein what is covered by these claims involves binding activity with proteins having at least three randomized loops. These claims now include a limitation equivalent to that of original claim 50, a claim that was free of the § 103 rejection. Indeed, nowhere in the cited references is the presently claimed invention taught or suggested.

The primary reference by Main provides the solution structure of the tenth type III module of fibronectin. The Office relies on this reference for the teaching that the FG

[1] Applicants note that Koide also indicates that the N-terminal tail, adjacent to the BC and FG loops in the three-dimensional Fn3 structure, might also be amenable to mutation; this sequence is not a loop structure.

loop of ^{10}Fn3 has a topology similar to that of an immunoglobulin C domain, and that this provides the motivation for combining this reference with Lee and Nygren, which discuss antibody molecules. On this issue, Applicants first point out that, while Main does indicate that the topology of ^{10}Fn3 is similar to an immunoglobulin C domain, Main also states that the topology of ^{10}Fn3 is actually closer to a number of non-immunoglobulin proteins, calling into question whether this reference can be used as the basis for a combination with Lee and Nygren. Specifically, the Office has not shown a motivation to combine this reference's information with references about antibodies. The teaching of Main would not drive one skilled in the art to focus on antibodies instead of non-immunoglobulin proteins.

Moreover, Main fails to provide many elements of Applicants' claims. Main never indicates that human fibronectin, or any of its modules, should be used as a scaffold for presenting multiple randomized loop sequences for the purpose of screening or detecting compounds not naturally bound by ^{10}FnIII. The teaching of Main is limited to an academic study focused entirely on attempts to understand how the fibronectin "RGD" motif mediates changes in cell adhesion activity and integrin specificity. It is in this context that Main states "The structure presented in this paper gives insight into the way a functional loop can be built onto a structural framework and, by virtue of its flexibility, be able to perform a wide range of functions." This statement, which is relied upon by the Office, is not an indication that the RGD sequence can be replaced by a

randomized loop. Quite to the contrary, it is an indication that the RGD sequence can play multiple roles, or — as stated in Main — it is an explanation of how "the interaction between fibronectin and its receptors can be finely tuned to encompass the wide range of integrin interactions among different cells" (page 676, col. 2, lines 17-20). Moreover, Main never provides a teaching that all three loops of the human ^{10}FnIII domain should or could be randomized. As acknowledged by the Office, Main also fails to teach the use of scaffold-based proteins that include such multiple randomized loops in screening or detection methods.

The secondary references provided in the Action do not remedy these deficiencies in the Main teaching. Lee discloses nothing about fibronectin scaffolds. Instead, Lee describes experiments in which the RGD binding motif is inserted into an immunoglobulin domain and its binding capacity examined. Based on these experiments, Lee states that this immunoglobulin construct may be used generally as a presentation scaffold. Lee does not indicate that fibronectin or its modules could be used as scaffolds. Lee never discloses a randomized fibronectin loop, and Lee certainly does not teach or suggest the use of scaffold-based proteins derived from the tenth module of the human fibronectin type III domain that include at least three randomized loops in screening or detection methods.

Finally, the reference by Nygren teaches a variety of scaffolds for engineering novel protein binding sites. This reference is relied upon for the teaching that "the use of

combinatorial approaches coupled with a powerful selection or screening strategy can be used to obtain novel proteins capable of binding a desired target molecule." While Applicants do not generally disagree with this statement, they point out that this Nygren passage begins by indicating that these approaches are applied "starting with a suitable protein domain." Nygren, like Lee, discusses the use of antibody scaffolds, as well as other scaffold systems, but Nygren does not mention fibronectin. Nygren, like the other references, also fails to suggest the randomization of multiple human ^{10}Fn3 loops or the use of scaffold-based proteins containing such loops in screening or detection assays.

Given these considerable deficiencies in the cited references, they cannot and do not form the basis for a *prima facie* case of obviousness as applied to the present claims. This rejection may be withdrawn.

Conclusion

Applicants submit that this case is now in condition for allowance, and such action is respectfully requested.

Applicants note that an Information Disclosure Statement was filed on May 11, 2000 and a Supplemental Information Disclosure Statement was filed on September 6, 2001. Applicants request that these Statements be reviewed by the Examiner, and the accompanying Forms PTO-1449 be initialed and returned with the next Action.

Enclosed is a petition to extend the period for replying for three months, to and

including March 7, 2002.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 7 March 2002

Karen L. Elbing, Ph.D.
Reg. No. 35,238

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

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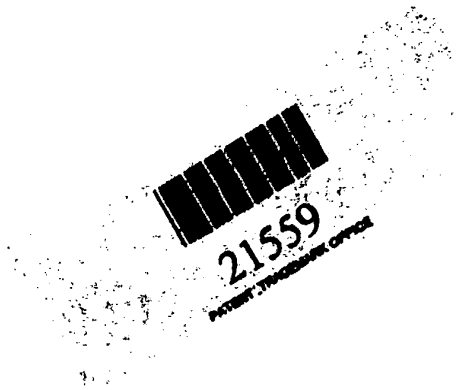

1: J Mol Biol 1998 Dec 11;284(4):1141-51

IDEAL

The fibronectin type III domain as a scaffold for novel binding proteins.

Koide A, Bailey CW, Huang X, Koide S.

Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, NY, 14642, USA.

The fibronectin type III domain (FN3) is a small autonomous folding unit which occurs in many animal proteins involving in ligand binding. The beta-sandwich structure of FN3 closely resembles that of immunoglobulin domains. We have prepared a phage display library of FN3 in which residues in two surface loops were randomized. We have selected mutant FN3s which bind to a test ligand, ubiquitin, with significant affinities, while the wild-type FN3 shows no measurable affinity. A dominant clone was expressed as a soluble protein and its properties were investigated in detail. Heteronuclear NMR characterization revealed that the selected mutant protein retains the global fold of FN3. It also has a modest conformational stability despite mutations at 12 out of 94 residues. These results clearly show the potential of FN3 as a scaffold for engineering novel binding proteins.

PMID: 9837732 [PubMed - indexed for MEDLINE]

MARKED UP VERSION TO SHOW AMENDMENTS MADE

In the Specification:

Replace the first paragraph on page 1 (lines 7-9) with the following amended paragraph:

> This application claims the benefit of the filing date of provisional application, U.S.S.N. 60/111,737, filed December 10, 1998, now abandoned, and is a continuation-in-part of utility application, U.S.S.N. 09/456,693, filed December 9, 1999.

Replace the first full paragraph on page 11 (lines 2-14) with the following amended paragraph:

> The present invention provides a number of advantages. For example, as described in more detail below, the present antibody mimics exhibit improved biophysical properties, such as stability under reducing conditions and solubility at high concentrations. In addition, these molecules may be readily expressed and folded in prokaryotic systems, such as E. coli, in eukaryotic systems, such as yeast, and in in vitro translation systems, such as the rabbit reticulocyte lysate system. Moreover, these molecules are extremely amenable to affinity maturation techniques involving multiple cycles of selection, including in vitro selection using RNA-protein fusion technology (Roberts and Szostak, Proc. Natl. Acad. Sci USA 94:12297, 1997; Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1 and U.S.S.N. 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al. WO98/31700), phage display (see, for example, Smith and Petrenko, Chem. Rev. 97:317, 1997), and yeast display systems (see, for example, Boder and Wittrup, Nature Biotech. 15:553, 1997).

Replace the third paragraph on page 12 (lines 7-11) with the following amended paragraph:

> FIGURE 5 is a photograph showing the structural similarities between a ^{10}Fn3 domain and 15 related proteins, including fibronectins, tenascins, collagens, and undulin. In this photograph, the regions are labeled as follows: constant, dark

blue; conserved, light blue; neutral, white; variable, red; and [RGB] RGD integrin-binding motif (variable), yellow.

Replace the fourth paragraph on page 12 (lines 12-16) with the following amended paragraph:

FIGURE 6 is a photograph showing space filling models of fibronectin III modules 9 and 10, in each of two different orientations. The two modules and the integrin binding loop [(RGB)] (RGD) are labeled. In this figure, blue indicates positively charged residues, red indicates negatively charged residues, and white indicates uncharged residues.

Replace the first paragraph on page 15 (lines 2-17) with the following amended paragraph:

The antibody mimics of the present invention are based on the structure of a fibronectin module of type III (Fn3), a common domain found in mammalian blood and structural proteins. This domain occurs more than 400 times in the protein sequence database and has been estimated to occur in 2% of the proteins sequenced to date, including fibronectins, [tenscin] tenascin, intracellular cytoskeletal proteins, and prokaryotic enzymes (Bork and Doolittle, Proc. Natl. Acad. Sci. USA 89:8990, 1992; Bork et al., Nature Biotech. 15:553, 1997; Meinke et al., J. Bacteriol. 175:1910, 1993; Watanabe et al., J. Biol. Chem. 265:15659, 1990). In particular, these scaffolds include, as templates, the tenth module of human Fn3 (^{10}Fn3), which comprises 94 amino acid residues. The overall fold of this domain is closely related to that of the smallest functional antibody fragment, the variable region of the heavy chain, which comprises the entire antigen recognition unit in camel and llama IgG (Figure 1, 2). The major differences between camel and llama domains and the ^{10}Fn3 domain are that (i) ^{10}Fn3 has fewer beta strands (seven vs. nine) and (ii) the two beta sheets packed against each other are connected by a disulfide bridge in the camel and llama domains, but not in ^{10}Fn3.

Replace the paragraph beginning on page 20, line 19 and ending on page 21, line 14 with the following amended paragraph:

The antibody mimics described herein may be used in any technique for evolving new or improved binding proteins. In one particular example, the target of binding is immobilized on a solid support, such as a column resin or microtiter plate well, and the target contacted with a library of candidate scaffold-based binding proteins. Such a library may consist of ^{10}Fn3 clones constructed from the wild type ^{10}Fn3 scaffold through randomization of the sequence and/or the length of the ^{10}Fn3 CDR-like loops. If desired, this library may be an RNA-protein fusion library generated, for example, by the techniques described in Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1, and 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al., WO98/31700; and Roberts & Szostak, Proc. Natl. Acad. Sci. USA (1997) vol. 94, p. 12297-12302. Alternatively, it may be a DNA-protein library (for example, as described in Lohse, DNA-Protein Fusions and Uses Thereof, U.S.S.N. 60/110,549, filed December 2, 1998, now abandoned, and 09/453,190, filed December 2, 1999). The fusion library is incubated with the immobilized target, the support is washed to remove non-specific binders, and the tightest binders are eluted under very stringent conditions and subjected to PCR to recover the sequence information or to create a new library of binders which may be used to repeat the selection process, with or without further mutagenesis of the sequence. A number of rounds of selection may be performed until binders of sufficient affinity for the antigen are obtained.

Replace the paragraph beginning on page 22, line 22 and ending on page 23, line 11 with the following amended paragraph:

A complex library was constructed from three DNA fragments, each of which contained one randomized area corresponding to a segment encoding a CDR-like loop. The fragments were named BC, DE, and FG, based on the names of the CDR-H-like loops [contained within] encoded by them; in addition to encoding ^{10}Fn3 sequence and a randomized sequence, each of the fragments contained stretches encoding an N-terminal His_6 domain or a C-terminal FLAG peptide tag. At each junction between two fragments (i.e., between the BC and DE fragments or between the DE and FG fragments), each DNA fragment contained recognition sequences for the EarI Type IIS restriction endonuclease. This restriction enzyme allowed the splicing together of adjacent fragments while removing all foreign, non-^{10}Fn3-encoding, sequences. It also allows for a recombination-like mixing of the three ^{10}Fn3-encoding fragments between cycles of mutagenesis and selection.

Replace the second paragraph on page 23 (lines 12-18) with the following amended paragraph:

Each DNA fragment was assembled from two overlapping oligonucleotides, which were first annealed, then extended to form the double-stranded DNA form of the fragment. The oligonucleotides that were used to construct and process the three fragments are listed below; the "Top" and "Bottom" species for each fragment are the oligonucleotides that contained the entire ^{10}Fn3 encoding sequence. In these oligonucleotides designations, "N" indicates A, T, C, or G; and "S" indicates C or G.

Replace the header, on page 23 (line 19) with the following amended header:

[HfnLbcTop (His):] HFnLBCTop(His):

Replace the header, on page 24 (line 1) with the following amended header:

[HfnLbcTop (an alternative N-terminus):] HfnLBCTop (an alternative N-terminus):

Replace the header, on page 25 (line 15) with the following amended header:

[T7Tmv] T7TMV (introduces T7 promoter and TMV untranslated region needed for in vitro translation):

Replace the first paragraph on page 26 (lines 2-4) with the following amended paragraph:

Unispl-s ([spint] splint oligonucleotide used to ligate mRNA to the puromycin-containing linker, described by Roberts et al, 1997, supra): 5'-TTTTTTTTTNAGCGGATGC-3' (SEQ ID NO: 13)

Replace the paragraph beginning on page 26, lines 14 and ending on page 27, line

4 with the following amended paragraph:

Next, each of the double-stranded DNA fragments was transformed into [a] an RNA-protein fusion [PROfusion™] using the technique developed by Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1 and U.S.S.N. 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al., WO98/31700; and Roberts & Szostak, Proc. Natl. Acad. Sci. USA (1997) vol. 94, p. 12297-12302. Briefly, the fragments were transcribed using an Ambion in vitro transcription kit, MEGAshortscript (Ambion, Austin, TX), and the resulting mRNA was gel-purified and ligated to a DNA-puromycin linker using DNA ligase. The mRNA-DNA-puromycin molecule was then translated using the Ambion rabbit reticulocyte lysate-based translation kit. The resulting mRNA-DNA-puromycin-protein [PROfusion™] fusion was purified using Oligo(dT) cellulose, and a complementary DNA strand was synthesized using reverse transcriptase and the RT primers described above (Unisplint-S or flagASA), following the manufacturer's instructions.

Replace the second paragraph on page 27 (lines 5-13) with the following amended paragraph:

The [PROfusion™] RNA-protein fusion obtained for each fragment was next purified on the resin appropriate to its peptide purification tag, i.e., on Ni-NTA agarose for the His_6-tag and M2 agarose for the FLAG-tag, following the procedure recommended by the manufacturer. The [DNA] cDNA component of the tag-binding [PROfusions™] RNA-protein fusions was amplified by PCR using Pharmacia Ready-to-Go PCR Beads, 10 pmol of 5' and 3' PCR primers, and the following PCR program (Pharmacia, Piscataway, NJ): Step 1: 95°C for 3 minutes; Step 2: 95°C for 30 seconds, 58/62°C for 30 seconds, 72°C for 1 minute, 20/25/30 cycles, as required; Step 3: 72°C for 5 minutes; Step 4: 4°C until end.

Replace the third paragraph on page 27 (lines 14-20) with the following amended paragraph:

The resulting amplified DNA was cleaved by 5 U EarI (New England Biolabs) per1 ug DNA; the reaction took place in T4 DNA Ligase Buffer (New England Biolabs) at 37°C, for 1 hour, and was followed by an incubation at 70°C

for15 minutes to inactivate Ear I. Equal amounts of the BC, DE, and FG DNA fragments were combined and ligated to form a full-length ^{10}Fn3 gene with randomized loops. The ligation required 10 U of fresh EarI (New England Biolabs) and 20 U of T4 DNA Ligase (Promega, Madison, WI), and took 1 hour at 37°C.

Replace the paragraph starting on page 27, line 21 and ending on page 28, line 3 with the following amended paragraph:

Three different DNA libraries were made in the manner described above. Each contained DNA encoding the form of the FG loop with 10 randomized residues. The DNA encoding the BC and the DE loops of the first library bore the wild type ^{10}Fn3 sequence; DNA encoding a BC loop with 7 randomized residues and a wild type DE loop made up the second library; and DNA encoding a BC loop with 7 randomized residues and a DE loop with 4 randomized residues made up the third library. The complexity of the DNA encoding the FG loop in each of these three libraries was 10^{13}; the further two randomized loops provided the potential for a complexity too large to be sampled in a laboratory.

Replace the first paragraph on page 28 (lines 4-9) with the following amended paragraph:

The three DNA libraries constructed were combined into one master library in order to simplify the selection process; target binding itself was expected to select the most suitable library for a particular challenge. [PROfusions™] RNA-protein fusions were obtained from the master DNA library following the general procedure described in Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1, and 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al., WO98/31700; and Roberts & Szostak, Proc. Natl. Acad. Sci. USA (1997) vol. 94, p. 12297-12302 (Figure 8).

Replace the second paragraph on page 28 (lines 11-19) with the following amended paragraph:

The master library in the [PROfusion™] RNA-protein fusion form was

subjected to selection for binding to TNF-α. Two protocols were employed: one in which the target was immobilized on an agarose column and one in which the target was immobilized on a BIACORE chip. First, an extensive optimization of conditions to minimize background binders to the agarose column yielded the favorable buffer conditions of 50 mM HEPES pH 7.4, 0.02% Triton, 100 μg/ml Sheared Salmon Sperm DNA. In this buffer, the non-specific binding of the ^{10}Fn3 [RNA] RNA-protein fusion to TNF-α Sepharose was 0.3%. The non-specific binding background of the ^{10}Fn3 [RNA-DNA] RNA-protein fusion to TNF-α Sepharose was found to be 0.1%.

Replace the third paragraph on page 28 (lines 20-24) with the following amended paragraph:

During each round of selection on TNF-α Sepharose, the [Profusion™] RNA-protein fusion library was first preincubated for an hour with underivatized Sepharose to remove any remaining non-specific binders; the flow-through from this pre-clearing was incubated for another hour with TNF-α Sepharose. The TNF-α Sepharose was washed for 3-30 minutes.

Replace the first paragraph on page 29 (lines 1-5) with the following amended paragraph:

After each selection, the [PROfusion™ DNA] cDNA from the RNA-protein fusion that had been eluted from the solid support with 0.3 M NaOH or 0.1M KOH was amplified by PCR; a DNA band of the expected size persisted through multiple rounds of selection (Figure 9); similar results were observed in the two alternative selection protocols, and only the data from the agarose column selection is shown in Figure 9.

Replace the second paragraph on page 29 (lines 6-10) with the following amended paragraph:

In the first seven rounds, the binding of library [PROfusions™] RNA-protein fusions to the target remained low; in contrast, when free protein was

translated from DNA pools at different stages of the selection, the proportion of the column binding species increased significantly between rounds (Figure 10). Similar selections may be carried out with any other binding species target (for example, IL-1 and IL-13).

Replace the third paragraph on page 29 (lines 12-19) with the following amended paragraph:

Wild-type ^{10}Fn3 contains an integrin-binding [tripepetide] tripeptide motif, Arginine 78 - Glycine 79 - Aspartate 80 (the "RGD motif') at the tip of the FG loop. In order to avoid integrin binding and a potential inflammatory response based on this tripeptide in vivo, a mutant form of ^{10}Fn3 was generated that contained an inert sequence, Serine 78 - Glycine 79 - Glutamate 80 (the "SGE mutant"), a sequence which is found in the closely related, wild-type ^{11}Fn3 domain. This SGE mutant was expressed as an N-terminally His_6-tagged, free protein in E. coli, and purified to homogeneity on a metal chelate column followed by a size exclusion column.

Replace the paragraph beginning on page 36, line 23 and ending at page 37, line 9 with the following amended paragraph:

In one exemplary use for fibronectin scaffold selection on chips, [an] a ^{10}Fn3-scaffold library-based selection was performed against TNF-α, using library of human ^{10}Fn3 variants with randomized loops BC, DE, and FG. The library was constructed from three DNA fragments, each of which contained nucleotide sequences that encoded approximately one third of human ^{10}Fn3, including one of the randomized loops. The DNA sequences that encoded the loop residues listed above were rebuilt by oligonucleotide synthesis, so that the codons for the residues of interest were replaced by (NNS)n, where N represents any of the four deoxyribonucleotides (A, C, G, or T), and S represents either C or G. The C-terminus of each fragment contained the sequence for the FLAG purification tag.

Replace the second paragraph on page 37 (lines 10-23) with the following

amended paragraph:

Once extended by Klenow, each DNA fragment was transcribed, and the transcript was ligated to a puromycin-containing DNA linker, and translated in vitro, as described by Szostak et al. (Roberts and Szostak, Proc. Natl. Acad. Sci USA 94:12297, 1997; Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1 and U.S.S.N. 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al., WO98/31700), to generate an mRNA-peptide fusion, which was then reverse-transcribed into a DNA-mRNA-peptide fusion. The binding of the FLAG-tagged peptide to M2 agarose separated full-length fusion molecules from those containing frameshifts or superfluous stop codons; the DNA associated with the purified full-length fusion was amplified by PCR, then the three DNA fragments were cut by Ear I restriction endonuclease and ligated to form the full length template. The template was transcribed, and the transcript was ligated to puromycin-containing DNA linkers, and translated to generate a [^{10}Fn3-PROfusion™] ^{10}Fn3-RNA-protein fusion library, which was then reverse-transcribed to yield the DNA-mRNA-peptide fusion library which was subsequently used in the selection.

Replace the third partial paragraph on page 37 (lines 24-25) with the following amended partial paragraph:

Selection for TNF-α binders took place in 50 mM HEPES, pH 7.4, 0.02% Triton-X, 0.1 mg/mL salmon sperm DNA. The [PROfusion™] RNA-protein fusion library was

Replace the first partial paragraph on page 38 (lines 1-4) with the following amended partial paragraph:

incubated with Sepharose-immobilized TNF-α; after washing, the DNA associated with the tightest binders was eluted with 0.1 M KOH, amplified by PCR, and transcribed, and the transcript ligated, translated, and reverse-transcribed into the starting material for the next round of selection.

Replace the second paragraph on page 38 (lines 5-8) with the following

amended partial paragraph:

Ten rounds of such selection were performed (as shown in Figure 13); they resulted in [a PROfusion™] an RNA-protein fusion pool that bound to TNF-α-Sepharose with the apparent average Kd of 120 nM. Specific clonal components of the pool that were characterized showed TNF-α binding in the range of 50-500 nM.

Replace the first partial paragraph on page 41 (lines 1-12) with the following amended partial paragraph:

[aminopropytrimethoxysilane] aminopropyltrimethoxysilane in 95% acetone / 5% water was prepared and allowed to hydrolyze for 20 minutes. The glass slides were immersed in the hydrolyzed silane solution for 5 minutes with gentle agitation. Excess silane was removed by subjecting the slides to ten 5-minute washes, using fresh portions of 95% acetone / 5% water for each wash, with gentle agitation. The slides were then cured by heating at 110°C for 20 minutes. The silane treated slides were immersed in a freshly prepared 0.2% solution of phenylene 1,4-diisothiocyanate in 90% DMF / 10% pyridine for two hours, with gentle agitation. The slides were washed sequentially with 90% DMF / 10% pyridine, methanol, and acetone. After air drying, the functionalized slides were stored at 0°C in a vacuum desiccator over anhydrous calcium sulfate. Similar results were obtained with commercial amine-reactive slides (3-D Link, Surmodics).

Replace the second paragraph on page 41 (lines 13-24) with the following amended paragraph:

Oligonucleotide capture probes were prepared with an automated DNA synthesizer (PE BioSystems Expedite 8909) using conventional phosphoramidite chemistry. All reagents were from Glen Research. Synthesis was initiated with a solid support bearing an orthogonally protected amino functionality, whereby the 3'-terminal amine is not unmasked until final deprotection step. The first four monomers to be added were hexaethylene oxide units, followed by the standard A, G, C and T monomers. All capture oligo sequences were cleaved from the solid support and deprotected with ammonium hydroxide, concentrated to [dyrness] dryness, precipitated in ethanol, and purified by reverse-phase HPLC using an

acetonitrile gradient in triethylammonium acetate buffer. Appropriate fractions from the HPLC were collected, evaporated to dryness in a vacuum centrifuge, and then coevaporated with a portion of water.

In the Claims

Claims 40-42, 51, 52, 57, 59-61, and 63-64 are amended.

40. (Amended) A method for obtaining a scaffold-based protein [which] that binds to a compound, said method comprising:

(a) contacting [said] a compound with a library of scaffold-based candidate proteins, [said candidate protein comprising a] wherein the scaffold is derived from the tenth module of the human fibronectin type III domain (^{10}Fn3), said scaffold-based proteins having at least one randomized loop, said library comprising scaffold-based proteins characterized by their ability to bind to compounds that are not bound by said human fibronectin type III domain and wherein said binding ability results from the randomization of said at least one loop, said contacting being carried out under conditions that allow binding to form a compound-scaffold-based protein complex [formation]; and

(b) obtaining, from said complex, [said] a scaffold-based protein having at least three randomized loops [which] that binds to said compound.

41. (Amended) A method for obtaining a compound [which] that binds to a scaffold-based protein, [said protein comprising a fibronectin type III domain having at

least one randomized loop], said method comprising:

(a) contacting [said] a scaffold-based protein with a candidate compound, wherein the scaffold is derived from the tenth module of the human fibronectin type III domain (^{10}Fn3), said scaffold-based protein having at least three randomized loops, said scaffold-based protein being characterized by its ability to bind to a compound that is not bound by said human fibronectin type III domain and wherein the binding ability results from the randomization of said at least three loops, said contacting being carried out under conditions that allow binding to form a compound-scaffold-based protein complex [formation]; and

(b) obtaining, from said complex, [said] a compound [which] that binds to said scaffold-based protein.

42. (Amended) The method of claim 40, said method further comprising further randomizing at least one loop of said human fibronectin type III domain of said protein obtained in step (b) and repeating said steps (a) and (b) using said further randomized protein.

51. (Amended) The method of claim [47] 40 or 41, wherein [the second loop of said ^{10}Fn3] at least one of said randomized loops is extended in length relative to the [naturally-occurring module] corresponding loop of human ^{10}Fn3.

52. (Amended) The method of claim [47] 40 or 41, wherein said ^{10}Fn3 lacks an integrin-binding motif.

57. (Amended) The method of claim 41, wherein said scaffold-based protein is immobilized on a solid support.

59. (Amended) A method for detecting a compound in a sample, said method comprising:

(a) contacting said sample with a scaffold-based protein which binds to said compound, wherein the scaffold is derived from the tenth module of the human [and which comprises a] fibronectin type III domain (^{10}Fn3), said scaffold-based protein having at least [one] three randomized loops, said scaffold-based protein being characterized by its ability to bind to a compound that is not bound by said human fibronectin type III domain and wherein the binding ability results from the randomization of said at least three loops, said contacting being carried out under conditions that allow binding to form a compound-scaffold-based protein complex [formation]; and

(b) detecting said complex, thereby detecting said compound in said sample.

60. (Amended) The method of claim 59, wherein said scaffold-based protein is

immobilized on a solid support.

61. (Amended) The method of claim 60, wherein said scaffold-based protein is immobilized on said solid support as part of an array.

63. (Amended) The method of claim 59, wherein said scaffold-based protein is covalently bound to a nucleic acid.

64. (Amended) The method of claim [59] 63, wherein said nucleic acid encodes said scaffold-based protein.

68. (New) The method of claim 40, wherein the proteins of said library have at least three randomized loops.